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COMMENTARY

Cheat invasion causes bacterial trait loss in lung infections

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Our traditional understanding of Darwin's principle of natural selection is that organisms become better adapted to the environment they live in. Accordingly, new traits are selected for because they increase the organisms' fitness. However, organisms cannot only gain but also lose traits. How can we understand trait loss in the light of natural selection? Darwin paid great attention to address this important issue (1). He argued that trait loss occurs because of disuse: organisms experience environmental changes, which might render previously beneficial traits useless, and since the maintenance of redundant traits is conceivable costly, natural selection should favor their degradation. Convincing support for Darwin's argument comes from studies on endosymbionts showing that shifts from free-living to endosymbiotic lifestyles are accompanied by large-scale genome reduction (2). Given this compelling evidence, one might think that alternative reasons for trait loss are quite implausible. Certainly, beneficial traits should not be lost, as this would curb the organisms' fitness. Yet exactly such scenarios exist, as evidenced by Andersen et al. (3) in their PNAS paper on evolutionary dynamics of the opportunistic human pathogen *Pseudomonas aeruginosa* during chronic lung infections.

Opportunistic pathogens typically experience drastic changes when colonizing the host from an environmental reservoir. When infections become chronic, pathogens can evolve within hosts and adapt to the new environment. Trait loss has been observed in such contexts, and following Darwin's logic, such losses have typically been attributed to disuse. However, Andersen et al. (3) argue that such conclusions are premature if the biological details of the

trait in question are not carefully considered. In their paper, Andersen et al. (3) studied the loss of pyoverdine production during long-term evolution of *P. aeruginosa* in cystic fibrosis lungs. Pyoverdine is a siderophore produced by this bacterium to scavenge insoluble or host-bound iron from the environment (4) (Fig. 1A). In the context of infections, it has been shown that pyoverdine is essential for bacteria to get hold of this trace element from host tissue (5, 6). But if pyoverdine is essential why is it then often lost during disease progression? Trapped with Darwin's disuse argument, the explanation most commonly put forward is that pyoverdine is no longer needed in chronic lung infections either because: (i) the prevailing low oxygen availability increases the concentration of readily available ferrous iron (7); (ii) iron availability is increased due to disease-related tissue damage (8); or (iii) other sources of iron, bound to citrate and heme, become available, for which *P. aeruginosa* has specific receptors for uptake (4).

Andersen et al. (3) were not convinced by this reasoning and conducted a series of experiments to contrast the loss-due-to-disuse hypothesis with an alternative evolutionary scenario, which is based on the idea that competition between strains can drive the loss of beneficial traits (Fig. 1B+C). This alternative hypothesis rests upon the observation that pyoverdine enacts its role outside the cell. Pyoverdine-producing individuals must therefore secrete their siderophores into the extra-cellular environment, thereby creating a public good, a pool of pyoverdine molecules that can be used by all individuals having the specific receptor for uptake (9). However, public good production is vulnerable to exploitation by cheating mutants that no longer produce but still capitalize on the pyoverdine secreted by others. By refraining from paying the cost of public good production, cheats can enjoy relative fitness advantages, and might therefore drive the cooperative trait to extinction (9). This scenario has at least been observed in evolving *P. aeruginosa* communities under laboratory conditions. Does it also represent a realistic scenario to explain trait loss in infections?

This is the question Andersen et al. (3) set out to test. The difficulty is how to discern between the two opposing hypotheses in a complex, uncontrollable environment such as the cystic fibrosis lung? The elegance of Andersen's et al. (3) approach resides in its simplicity. The authors analyzed genome sequences of *P. aeruginosa* isolates, collected over time from 60 cystic fibrosis patients. For each isolate, they phenotypically assessed its ability to produce pyoverdine, and scored whether mutations occurred in genes involved in pyoverdine synthesis and/or uptake. With this approach, Andersen et al. (3) could reconstruct the evolutionary history of *P. aeruginosa* within patients, and thereby test the contrasting predictions of the two hypotheses. The loss-due-to-disuse hypothesis predicts mutations to accumulate in genes involved in both pyoverdine synthesis and uptake, as any of these mutations would contribute to the deterioration of the redundant trait, and therefore confer fitness benefits. Conversely, the loss-due-to-cheating hypothesis predicts that mutations should exclusively occur in pyoverdine synthesis genes but not in the receptor gene, at least as long as pyoverdine producers and non-producers co-exist within a patient. This is because cheats can only spread and exploit producers if they maintain a functional receptor.

The data yielded strong support for the loss-due-to-cheating hypothesis. Andersen et al. (3) found that many isolates lost the ability to produce pyoverdine during the course of the infection, which went along with a significant accumulation of mutations in *pvdS*, a gene coding for the sigma factor controlling pyoverdine synthesis. Intriguingly, pyoverdine non-producers kept their receptors intact for several years, as long as producer strains co-existed in the same patient. Only after producers have vanished altogether, mutations also accumulated in the receptor gene. These reconstructed evolutionary trajectories combined with fitness assays, confirming that non-producers can indeed benefit from exogenously added pyoverdine, strongly suggest that pyoverdine trait loss is driven by cheating and not disuse in this system.

Exciting as these findings are, a key question that immediately arises is whether trait loss due to cheating is a common phenomenon in natural populations of bacteria or whether the reported example represents a special case. While no definite answer can be given at this stage, we certainly know that most bacterial species rely on a broad range of secreted compounds to scavenge resources from the environment, but also to build up biofilms, to move across surfaces and to fight competitors (10). Cheats have been shown to arise for many of these traits in laboratory settings, but also in infectious contexts (11-14). Thus, the loss of beneficial traits due to strain competition might be far more common then previously thought, and emerges as a valid alternative to Darwin's disuse argument.

A second important aspect to address is whether the understanding of selection pressures driving trait loss primarily excites evolutionary biologists or whether this phenomenon is of broader relevance. Or asked differently, why should patients and clinicians care about whether disease-causing virulence factors are lost due to disuse or cheating? This is a valid question, because cystic fibrosis is after all a serious disease, and we would hope that improved understanding of fundamental evolutionary aspects should also benefit patients in one or the other way. Indeed, there is a bigger scope for Andersen's et al. (3) findings, because the loss of beneficial virulence factors due to cheating is expected to compromise both the harm bacteria can inflict on the host, and the overall fitness of the bacterial collective (11, 15). In other words, the spreading of cheats should ease the burden of disease. By demonstrating that cheats can indeed spread in chronic infections, Andersen's et al. (3) have now set the foundation for novel antibacterial therapies, such as the modification of the host environment to accelerate the spread of cheats (16), or the engineering of antibiotic-susceptible cheats, that are introduced into established infections to first replace the pathogenic bacteria by cheating, and then to be eradicated themselves by antibiotic treatment (17).

To conclude, we can speculate about whether pyoverdine cheat invasion necessarily eases the burden of disease in cystic fibrosis lungs. This question is far from straightforward to answer, especially for a versatile pathogen like *P. aeruginosa*. One complication is that this bacterium possesses a second siderophore, called pyochelin, which is typically silent when pyoverdine is produced, but becomes expressed in pyoverdine non-producers (18). Can pyochelin compensate for the loss of pyoverdine? If so, then the harm to the patient might not be lowered when pyoverdine cheats spread. Another complication is that *P. aeruginosa* can tap other iron resources, such as ferrous iron bound to heme. Indeed, it has been shown that *P. aeruginosa* streamlines iron uptake via this channel over evolutionary time in the lung, an adaptation that coincides with the decline in pyoverdine availability (19). Finally, patients differ from one another with respect to the disease causing mutation in the CFTR gene, but also in their overall physiological status (20). Such inter-individual differences could affect bacterial evolution and potentially explain why pyoverdine was lost in many but not all patients. Taken together, the enigma on iron acquisition and pathogen evolution in the cystic fibrosis lung is complex and not yet fully resolved, but Andersen et al. (3) have unraveled a key piece of the puzzle.

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Fig. 1. (A) Microcolony of *P. aeruginosa* producing the auto-fluorescent pyoverdine (visible in the periplasma) to scavenge iron from the environment (image courtesy of Konstanze Schiessl). This trait is lost during chronic infections in lungs of cystic fibrosis patients. Two hypotheses have been proposed to explain this loss. Pyoverdine is selected against because: (B) iron becomes more readily available in the lung and pyoverdine is no longer needed for uptake; or (C) pyoverdine is still useful, but is exploited by cheating mutants that no longer produce but capitalize on the publically available pyoverdine secreted by others. Color code: red = iron, grey = host tissue, orange = pyoverdine, green = pyoverdine producer, blue = pyoverdine non-producer.